Chapter 16
Fluoroquinolone Resistance in Bacteria

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1 Introduction

Quinolones are some of the most widely prescribed antimicrobial agents in the world. For example, levofloxacin sales totaled $1.5 billion in 2005 (Johnson & Johnson Annual Report for 2005; http://www.investor.jnj.com). A detailed discussion of structure-activity relationships is beyond the scope of this chapter, but these agents have undergone several iterations, or "generations," which have consisted of structural modifications to improve potency and spectrum of activity. The first-generation quinolone upon which all subsequent derivatives are based is nalidixic acid (Fig. 1), which was isolated as a by-product during chloroquine synthesis (1). Nalidixic acid actually is a naphthyridone based on the presence of a nitrogen atom at position 8, whereas quinolones generally have a carbon atom at this position. Second-generation drugs, all of which have a fluorine at position 6 of the quinolone nucleus, include norfloxacin, ciprofloxacin, enoxacin, ofloxacin, and pefloxacin and third-generation agents include temafloxacin, levofloxacin, trovafloxacin, gatifloxacin, and moxifloxacin.

Many quinolones have been approved by various regulatory agencies worldwide and some have been withdrawn after widespread use revealed unforeseen toxicities. Examples of this include temafloxacin, which was found to be associated with hypoglycemia and hemolytic-uremic syndrome and trovafloxacin, found to be associated with severe hepatotoxicity (2, 3). Although serious adverse events following quinolone use are relatively rare, some that have been associated with these drugs include prolongation of the QTc interval which can predispose to serious, life-threatening arrhythmias, rash, seizure, glucose intolerance and, as already mentioned, hepatotoxicity (4).

Quinolones are broad-spectrum bactericidal agents active against many Gram-positive and Gram-negative bacteria that target the essential bacterial enzymes DNA gyrase and DNA topoisomerase IV (5). These enzymes are involved in DNA replication and repair and in the presence of a quinolone an intermediate ternary complex consisting of drug, enzyme, and a severed DNA strand is formed. These complexes block further DNA replication leading to cell death. Mutational alterations of the genes encoding DNA gyrase and/or topoisomerase IV in the so-called quinolone resistance determining region, or QRDR, and resulting in critical amino acid substitutions reduce quinolone interaction with each enzyme. These mutations are the basis for high-level, target-based quinolone resistance and will be discussed in detail later in this chapter. Another important mechanism of quinolone resistance is overexpression of membrane-based drug efflux pumps, which also will be discussed subsequently. Such efflux pumps reduce the effective intracellular drug concentration to either a non-inhibitory or borderline inhibitory level, favoring the emergence of target-based mutations and high-level resistance (6–8).

All clinically relevant bacterial species are capable of developing resistance to quinolones, but historically problematic organisms have been *Staphylococcus aureus* and *Pseudomonas aeruginosa*. For many quinolones these organisms tend to have a narrower therapeutic index than other bacteria in that the minimum inhibitory concentration (MIC) and achievable serum levels are relatively close. In this situation subtherapeutic drug levels will exist for long periods of time during therapy, favoring the emergence of point mutations in topoisomerase genes leading to reduced quinolone susceptibility. Newer agents with increased potency against *S. aureus* have helped to reduce this problem but unfortunately the majority of methicillin-resistant *S. aureus* (MRSA) strains recovered from clinical specimens in many areas of the world are already highly quinolone resistant, mainly on the basis of target mutations. Resistance in methicillin-susceptible (MSSA) strains is less problematic, but can be significant in some geographic locales. Over a 7-month period during 2005 we collected more than 200 bloodstream isolates of *S. aureus* from different patients hospitalized in Detroit, Michigan. Of these strains, 65% were MRSA and 35% were MSSA.

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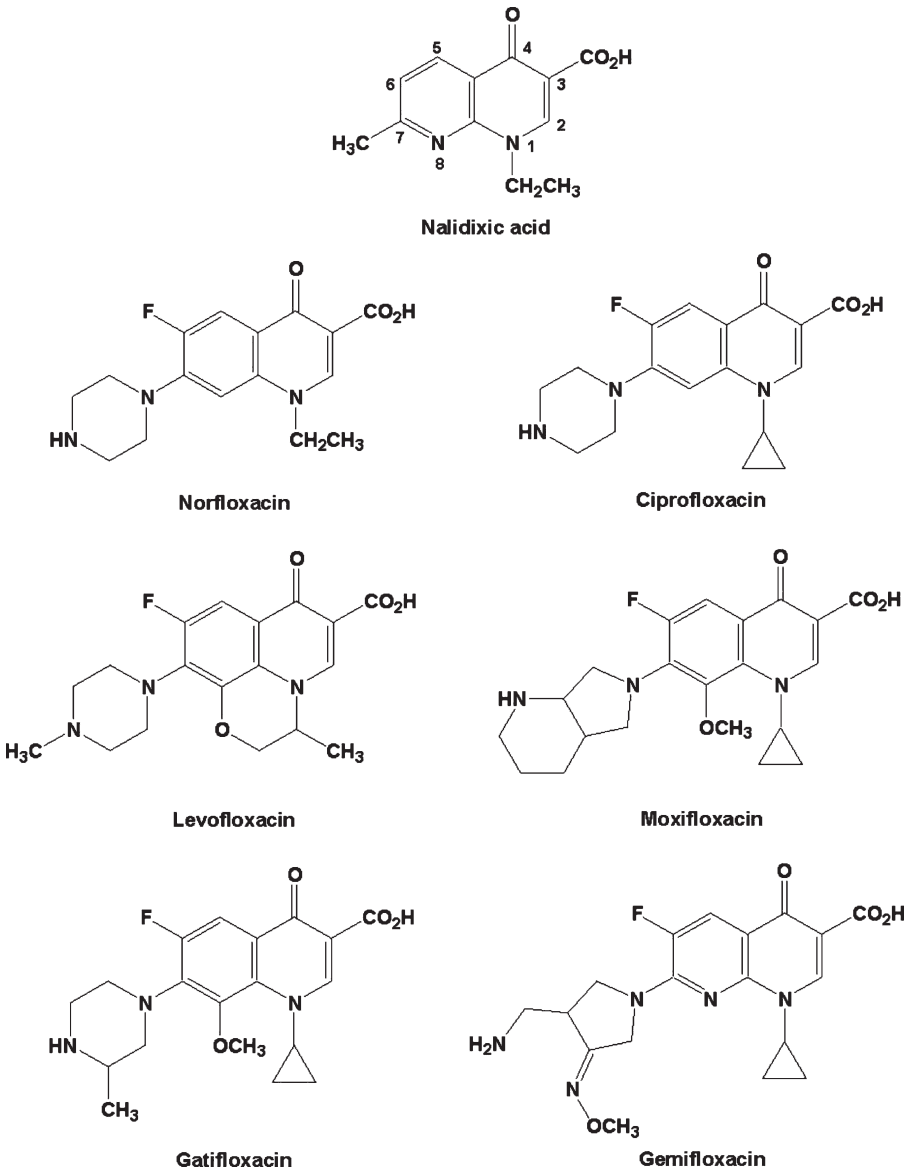

Fig. 1 Structures of selected quinolones. The numbering scheme of the quinolone nucleus is given for nalidixic acid

Norfloxacin resistance (MIC ≥ 16 μg/mL) was observed in 60 and 12% of MRSA and MSSA, respectively (unpublished data). Resistance rates for other areas may differ, but these data illustrate the extent of the problem in *S. aureus*.

In this chapter we will discuss quinolone resistance in both Gram-negative and Gram-positive bacteria. We will not address resistance to these agents in *Mycobacterium tuberculosis* since they are not frequently used to treat infections caused by this organism. However, many of the mechanisms that will be discussed here have also been found to exist in *M. tuberculosis* (9, 10). We will conclude with a short discussion on the means to limit quinolone resistance and perhaps to overcome some pre-existent resistance by use of efflux pump inhibitors.

2 Gram-Negative Bacteria

Gram-negative bacteria are an important cause of morbidity and mortality. The increasing antibacterial resistance observed in many Gram-negative organisms parallels the increasing use and abuse of antimicrobial agents, and this is certainly true for the quinolones (11). Until 1998 it was thought that quinolone resistance in Gram-negatives occurred either by way of target alteration or active drug extrusion by membrane-based efflux pumps. A third mechanism described more recently involves the Qnr protein, the gene for which is plasmid-encoded and thus transferable (12). This mechanism of quinolone resistance is addressed in detail elsewhere in this volume and will only be briefly described in this chapter.

2.1 Target-Mediated Resistance

As already mentioned, the targets of quinolones are the essential bacterial enzymes: DNA gyrase and topoisomerase IV. DNA gyrase, the major type II topoisomerase in bacteria and initially described by Gellert et al. is a heterotetramer composed of two pairs of subunits (A and B) encoded by the *gyrA* and *gyrB* genes, respectively (13). The GyrA subunits bind to DNA and the GyrB subunits are ATPases. The main function of this enzyme is to maintain negative supercoiling via DNA strand breakage and rejoining, a function that facilitates the movement of DNA through replication and transcription complexes. Negative supercoiling is essential for initiation of DNA replication and introduction of supercoils depends on the binding of ATP to gyrase with subsequent ATP hydrolysis (14). Thus, this process is sensitive to changes in membrane energetics. DNA gyrase also helps remove knots and in the bending and folding of DNA. Following the discovery of DNA gyrase, it was ascertained that this enzyme is a target of quinolones (15).

Kato et al. discovered DNA topoisomerase IV, a heterotetrameric enzyme composed of two subunit pairs encoded by the *parC* and *parE* genes (16). ParC and ParE are homologous with GyrA and GyrB, respectively, with a high degree of amino acid conservation in the QRDR regions. The principle function of topoisomerase IV appears to be its ability to decatenate linked daughter chromosomes at the terminal stages of DNA replication (17). Despite DNA gyrase and topoisomerase IV sharing considerable amino acid sequence similarity, they have distinct mechanisms of action. One of the important differences seems to be that DNA gyrase wraps DNA around itself, while topoisomerase IV does not (18). Given the homology between DNA gyrase and topoisomerase IV the latter enzyme was thought to also be a quinolone target, which has now been demonstrated clearly (19, 20).

As mentioned previously, quinolones bind to DNA-DNA gyrase and DNA-topoisomerase IV complexes and cause a conformational change in the enzyme structure (21, 22). They also alter the enzyme-bound DNA itself (23, 24). In the presence of quinolones the topoisomerases become trapped on DNA and the resultant quinolone-enzyme-DNA ternary complex forms a physical barrier at the replication fork, inhibiting further DNA replication which results in cell death (25).

In Gram-negative bacteria the primary target for most quinolones is DNA gyrase, with topoisomerase IV being a secondary target (19, 26). In contrast, in most Gram-positive bacteria and for most quinolones topoisomerase IV is the primary target (27, 28). These differences are thought to be due to the differential affinity of quinolones for the two enzymes in each respective background (29). Quinolone resistance occurs in a stepwise fashion as a result of the accumulation of mutations resulting in amino acid substitutions mainly in *gyrA* and *parC*. Less commonly, mutations occur in *gyrB* and *parE* that can contribute to reduced quinolone susceptibility (26, 30). Additional MIC increases are seen when a "first-step" mutant, having a critical amino acid substitution in the primary target, acquires a "second-step" mutation resulting in an amino acid substitution in the secondary target enzyme. Many topoisomerase mutations in *E. coli*, as well as many other Gram-negative bacteria, have been shown to correlate with raised quinolone MICs (Table 1).

Analyses of *gyrA* mutants have revealed that most of the quinolone-resistance conferring mutations cluster near the 5′ end of the gene in the QRDR region. For *E. coli*, this region includes codons 67–106 and for other species the region homologous to this (30, 31). Very near the QRDR is the codon for the active site tyrosine (codon 122). Tyrosine-122 binds covalently to DNA when the enzyme breaks the phosphodiester bonds of DNA, forming a phosphotyrosine linkage (32). Single *gyrB* mutants appear to be less resistant to quinolones than single *gyrA* mutants. In *E. coli* only two *gyrB* mutations have been recognized (Table 2). Only Asp426→Asn confers resistance to quinolones, whereas Lys447→Glu results in an increase in quinolone susceptibility (31).

Within topoisomerase IV, mutations in *parC* occur more frequently than those in *parE*. As mentioned previously, topoisomerase IV generally is the secondary quinolone target in *E. coli* and other Gram-negative organisms. g*yrA-parC* double mutants exhibit a higher level of quinolone resistance than *gyrA* single mutants, with the highest levels of resistance found in the mutants with two *gyrA* and two *parC* mutations. The reverse generally is true in Gram-positive organisms, where the first mutations are usually seen in the topoisomerase IV genes with the gyrase genes being the secondary targets.

Table 1 Topoisomerase amino acid substitutions associated with reduced quinolone susceptibility in *E. coli*

GyrA	GyrB	ParC	ParE
Ala51→Val	Asp426→Asn	Gly78→Asp	Leu445→His
Ala67→Ser	Lys447→Glu	Ser80→Arg, Ile, Leu	
Gly81→Cys, Asp		Glu84→Gly, Lys, Val	
Asp82→Gly			
Ser83→Leu, Trp, Ala, Val			
Ala84→Pro, Val			
Asp87→Ala, Asn, Gly, His, Tyr, Val			
Gln106→Arg, His			

Data are from (30, 31)

Table 2 Topoisomerase amino acid substitutions associated with reduced quinolone susceptibility in *S. aureus*

GyrA	GyrB	ParC (GrlA)	ParE (GrlB)
Ser84→Ala, Leu, Lys, Val	Asp437→Asn	Lys23→Asn	Pro25→His
Ser85→Pro	Arg458→Glu	Val41→Gly	Ser410→Pro
Glu86→ Lys, Gly	Glu477→Ala	Arg43→Cys	Glu422→Asp
Glu88→Lys, Val		Ile45→Met	Asp432→Asn, Gly, Val
Gly106→Asp		Ala48→Thr	Pro451→Gln, Ser
		Ser52→Arg	Asn470→Asp
		Asp69→Tyr	Glu472→Lys, Val
		Gly78→Cys	His478→Tyr
		Ser80→Phe, Tyr	
		Ser81→Pro	
		Glu84→Ala, Gly, Leu, Lys, Tyr, Val	
		His103→Tyr	
		Ala116→Glu, Pro	
		Pro157→Leu	
		Ala176→Gly, Thr	

Data are from (30, 31)

2.2 Decreased Outer Membrane Permeability

Quinolones must traverse the outer membrane, periplasmic space, cell wall, and cytoplasmic membrane of Gram-negative organisms to reach their topoisomerase targets. The porous bacterial cell wall does not impede the diffusion of small molecules such as quinolones and will not be considered further. The outer membrane may provide a rather formidable barrier, however, and in conjunction with efflux pumps (see below) can result in significant quinolone resistance (30, 31). Quinolones traverse this structure by two mechanisms, which include diffusion across the lipid bilayer and passage through pore-forming proteins called porins. Porins are protein channels that allow influx and egress of hydrophilic molecules. All quinolones may cross the outer membrane through the porins, but diffusion across the lipid bilayer is dependent on the hydrophobicity of the molecule. The more hydrophobic quinolones such as nalidixic acid are capable of traversing the lipid bilayer, whereas the more hydrophilic compounds such as ciprofloxacin are more dependent on porins (33, 34). Three main porins are found in *E. coli* and consist of OmpF, OmpC, and OmpA. Loss of porins by mutational inactivation of structural genes often manifests as a decrease in quinolone susceptibility, but this effect is significantly amplified in the presence of drug efflux. *E. coli* mutants with reduced amounts of OmpF, the most abundant porin, exhibit low-level quinolone resistance (35). Other unrelated drugs such as tetracyclines, chloramphenicol, and some β-lactams also utilize this porin and hence OmpF-deficient mutants also demonstrate resistance to these other agents due to decreased drug accumulation (36). Chromosomal loci such as *marRAB* and *soxRS* encode transcriptional factors that regulate OmpF expression in *E. coli* (37). Overexpression of *marA* and *soxS* results in post-transcriptional repression of OmpF and thus quinolone resistance by increasing the expression of *micF*, an antisense regulator (37, 38). More on the roles of MarA and SoxS in quinolone resistance will be presented in the next section.

The permeability of the outer membranes of *Pseudomonas aeruginosa* and *Acinetobacter baumanii* may account for some of their intrinsic resistance to various antibiotics, including quinolones. The *P. aeruginosa* outer membrane has very poor permeability to hydrophilic molecules, approximately 100-fold less than that of the *E. coli* outer membrane (39).

2.3 Efflux-Related Resistance

Gram-negative bacteria tend to be resistant to a wider range of antimicrobial agents compared to Gram-positive species. The outer membrane is one reason for this in that it acts as a barrier to the penetration of hydrophilic molecules. This mechanism generally confers only low-level reduced susceptibility. Membrane-based efflux pumps contribute more significantly to innate drug insensitivity. Bacterial efflux pumps can be divided into five families based on structural characteristics, mechanisms of action and source of energy for the transport process. These include primary transporters that depend on ATP hydrolysis for drug export (ATP-binding cassette, or ABC pumps) and secondary transporters that require an intact proton motive force (pmf) across the cell membrane for their function (major facilitator superfamily [MFS], resistance-nodulation-division [RND], small multidrug resistance [SMR], and the multidrug and toxic compound extrusion [MATE] families) (Fig. 2). Efflux pumps may be quite specific with respect to substrates transported, with a clinically relevant example being the various MFS tetracycline efflux pumps found in Gram-negative and Gram-positive bacteria (40). Circumvention of the resistance generated by specific drug pumps is as simple as providing alternative therapy with agents not affected by the pump in question. Multidrug efflux pumps, which have an apparent lack in substrate specificity, are capable of extruding numerous structurally dissimilar compounds, creating a multidrug resistant (MDR) phenotype, and can pose a very formidable therapeutic challenge (Table 3). Drug efflux can lead to subtherapeutic intracellular concentrations of an antibiotic substrate, thereby setting up the ideal milieu for the development of chromosomal mutations that confer high-level antibiotic resistance.

Efflux-related quinolone resistance has been identified in virtually all medically important Gram-negative organisms,

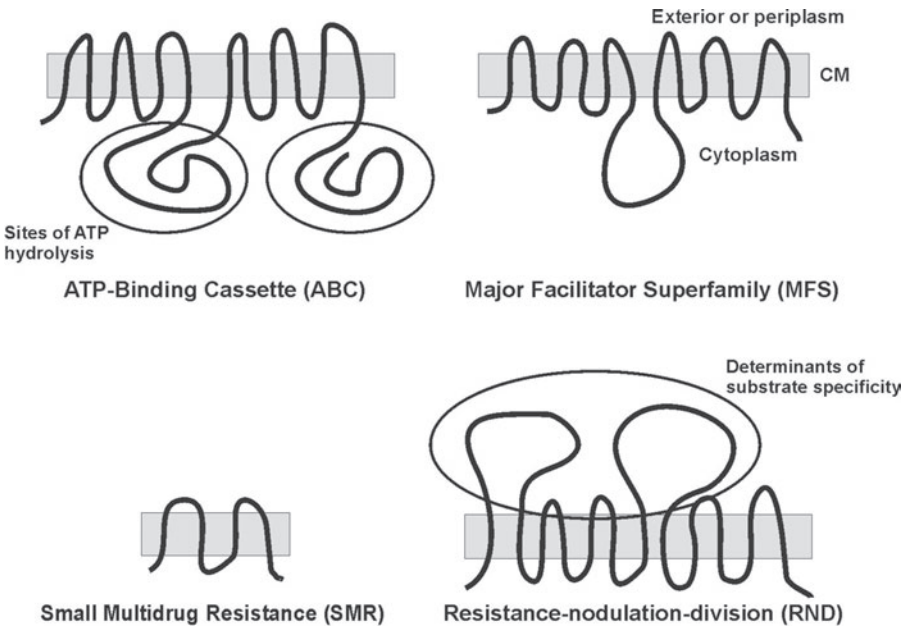

Fig. 2 Schematic illustrating the general structural characteristics of each family of bacterial efflux pump. The sites at which ATP hydrolysis occurs in ABC pumps are indicated. MATE pumps do not necessarily have the large central loop that is characteristic of members of the MFS and some MFS proteins have 14 membrane-spanning segments. Substrate specificity for RND pumps such as AcrB and perhaps other pumps of this family lie in the two large periplasmic loops. The cytoplasmic membrane is shown in *gray* and the cytoplasm and exterior/periplasm are as indicated

Table 3 Selected bacterial multidrug efflux pumps

Pump	Family	Organism	Selected substrates[a]
Gram-negative			
AcrB	RND	*E. coli*	FQ, BL, CM, TCN, TI
MdfA	MFS	*E. coli*	FQ, CM, EM, TCN
MexB	RND	*P. aeruginosa*	FQ, BL, CM, TCN, TI, TM
MexD	RND	*P. aeruginosa*	FQ, CM, EM, TCN, TI, TM
MexF	RND	*P. aeruginosa*	FQ, CM, TM
MexY	RND	*P. aeruginosa*	FQ, AF, AG, EB, EM
SmeE	RND	*S. maltophilia*	FQ, CM, TCN
NorM	MATE	*V. parahaemolyticus*	FQ, EB
Gram-positive			
NorA	MFS	*S. aureus*	FQ, AF, BAC, CT, EB, TPP
PmrA	MFS	*S. pneumoniae*	FQ, EB
Bmr	MFS	*B. subtilis*	FQ, AF, EB, TPP
Blt	MFS	*B. subtilis*	FQ, AF, EB, TPP
MepA	MATE	*S. aureus*	FQ, BAC, DQ, EB, TPP, PT
LmrA	ABC	*L. lactis*	FQ, AG, BL, CM, TCN

[a]*AF* acriflavine; *AG* aminoglycosides; *BAC* benzalkonium chloride; *BL* beta-lactams; *CM* chloramphenicol; *CT* cetrimide; *DQ* dequalinium; *EB* ethidium bromide; *EM* erythromycin; *FQ* fluoroquinolones; *PT* pentamidine; *TCN* tetracycline; *TI* tigecycline; *TM* trimethoprim; *TPP* tetraphenylphosphonium

including *E. coli* and *P. aeruginosa* (41). Pump-related resistance to quinolones is due to the activity of multidrug pumps; no pumps having quinolones as sole substrates have been described. These pumps are capable of transporting several antimicrobial agents and thus cross-resistance to multiple antibiotics can be observed in their presence. It has already been mentioned that the relatively impermeable Gram-negative outer membrane, which limits drug entry, works synergistically with efflux pumps capable of extruding drugs that do gain access to the cytoplasmic membrane (30, 31). The RND-type pumps of Gram-negative organisms are composed of three different subunits, which include the pump protein itself, which is a transmembrane protein having 12 membrane-spanning alpha helices or transmembrane segments (TMS), an outer membrane pore-forming channel or porin, and a periplasmic membrane fusion protein (MFP) that links the other two.

The AcrB pump is the predominant quinolone efflux system of *E. coli* (42). This pump is a member of the RND family and utilizes TolC as its outer membrane channel, to which it is associated by the AcrA MFP (43). AcrB has a broad substrate profile including quinolones, tetracyclines, chloramphenicol, ampicillin, nalidixic acid, rifampin and dyes and disinfectants. The expression of *acrAB*, which is transcribed as an operon, is governed by at least two global regulatory systems, the *marRAB* and *soxRS* loci; both systems positively regulate the production of AcrAB. Multiple antibiotic resistant (Mar-type) mutants of *E. coli* have mutations in the *marRAB* operon (44). The Mar phenotype is induced following exposure to a variety of chemicals with aromatic rings, including salicylate. The most common location for mutations conferring the Mar phenotype is in *marR*, which encodes for the repressor of the *marRAB* operon. *E.coli soxRS* mutants exhibit a similar resistance phenotype to *marR* mutants. Increased quantities of MarA and SoxS upregulate *acrAB* and down-regulate the production of the OmpF porin

channel. These changes lead to multiple antibiotic resistance by these synergistic mechanisms.

The crystal structure of AcrB in the presence and absence of substrates was recently solved (45). These data indicate that the pump acquires substrates from the outer leaflet of the cytoplasmic membrane; however, acquisition of substrate from the cytoplasm may also occur. Substrate specificity of AcrB seems to lie in its large periplasmic loops (Fig. 2) (46).

In *P. aeruginosa* the main multidrug efflux system (including quinolones) is the *mexAB-oprM* operon, which encodes proteins homologous to AcrAB-TolC in *E. coli. mexCD-oprJ*, *mexEF-oprN,* and *mexXY-oprM* are three additional multidrug resistance operons found in *P. aeruginosa*. Each of these operons encodes for a set of three proteins similar in structure and function to MexAB-OprM and all are RND type efflux pumps. Like AcrAB-TolC, the most striking characteristic of these pump systems is their broad substrate specificity. The substrate profile for MexAB-OprM includes quinolones, chloramphenicol, nalidixic acid, trimethoprim, tetracyclines (including tigecycline), dyes, disinfectants and organic solvents (Table 3). Most wild-type strains of *P. aeruginosa* express MexAB-OprM constitutively, which contributes to the intrinsic multidrug resistant nature of this organism (47). Overexpression of efflux pumps due to chromosomal mutations in the promoter region of the pump genes or in the gene encoding the regulator for pump gene expression can cause clinically relevant resistance to antimicrobial agent substrates. There are a number of laboratories actively searching for compounds capable of efflux pump inhibition, which could restore clinically relevant activity of substrate antibiotics.

Multidrug efflux pumps having quinolones as substrates have been identified in many other Gram-negative bacteria. Examples include the SmeDEF RND pump system of *Stenotrophomonas maltophilia*, the NorM and BexA MATE pumps of *Vibrio parahaemolyticus* and *Bacteroides thetaiotaomicron*, respectively, and the VceAB MFS pump of *V. cholerae* (48–51) (Table 3). Overexpression of these pumps in either their natural or a heterologous background results in increased MICs for a variety of quinolones.

2.4 Plasmid-Mediated Quinolone Resistance

In 1998 Martínez-Martínez et al. reported quinolone resistance to be expressed in the presence of pMG252, a plasmid belonging to incompatibility group IncC (12). This plasmid mediates low-level quinolone resistance (to both nalidixic acid and more modern quinolones) and has a broad host range. Subsequently, the gene responsible for quinolone resistance was identified and named *qnr* (52). Qnr "protects" both DNA gyrase and topoisomerase IV from quinolone inhibition (52–54). Prevalence studies have revealed that among quinolone-resistant strains of *E. coli* recovered in Shanghai, China, 7.7% contained the *qnrA* gene (55). In the United States, *qnrA* was present in 11.1% of quinolone-resistant *Klebsiella pneumoniae* strains but not in any of the tested *E. coli* strains (56). Further investigation led to the discovery that *qnrA* was present in clinical strains of *Enterobacter* spp. (57). Thus, the *qnrA* gene is widely distributed and contributes to quinolone resistance in Enterobacteriaceae. More recently, a new *qnrA*-related gene called *qnrB* was discovered in a strain of *K. pneumoniae* that had less than 40% amino acid sequence identity with *qnrA* (58). Although *qnr* confers relatively low-level quinolone resistance, its presence may facilitate selection of other quinolone mutations leading to high-level resistance. Further discussion of this novel quinolone resistance mechanism can be found elsewhere in this volume.

2.5 Enzymatic Modification of Quinolones

Being synthetic substances, the occurrence of natural degradation systems in bacteria seemed unlikely. However, fungi capable of degrading ciprofloxacin and the veterinary fluoroquinolone enrofloxacin have been identified (59, 60). Recently, a plasmid-associated gene recovered from a clinical *E. coli* strain was found to encode an aminoglycoside acetyltransferase that could also acetylate selected fluoroquinolones and compromise their antimicrobial activity (61). The effect of acetylation was relatively small, as exemplified by expressing the gene in question (aac[6′]-Ib-cr) from a plasmid in an *E. coli* background. Norfloxacin and ciprofloxacin MICs were increased fourfold, whereas those of levofloxacin and gemifloxacin were unaffected. The MIC increases were not clinically significant, but the existence of a plasmid-based and naturally occurring enzyme capable of modifying quinolones is worrisome as widespread dissemination is possible. The combination of this resistance mechanism with others causing borderline MIC increases, such as efflux pumps or single QRDR mutations, may result in a clinically relevant fully resistant organism.

3 Gram-Positive Bacteria

Fewer quinolone resistance mechanisms are found in Gram-positive bacteria than those identified in Gram-negatives. The lack of an outer membrane results in no permeability issues beyond those posed by the cytoplasmic membrane and no Qnr-like proteins or quinolone-modifying enzymes have been identified in this group of organisms. The mechanisms of quinolone resistance that have been recognized include target-based mutations and drug efflux. Studies done in vitro provide

evidence that inhibition of efflux pumps reduces the emergence of topoisomerase mutations in both *S. aureus* and *Streptococcus pneumoniae*, suggesting that efflux pumps play a critical role in the evolution of high-level quinolone resistance (6, 7).

3.1 Target-Mediated Resistance

Similar to the situation in Gram-negative bacteria, mutations in the QRDR regions of mainly *gyrA* and *parC (grlA* in *S. aureus)* resulting in amino acid substitutions is the main mechanism of quinolone resistance in Gram-positive bacteria. In general, GrlA is the primary quinolone target in Gram-positives and single amino acid substitutions in this enzyme can result in clinically relevant resistance (62). Accumulation of QRDR mutations first in *parC* and then in *gyrA* typically results in very high MICs. Topoisomerase amino acid substitutions correlating with quinolone resistance in *S. aureus* are presented in Table 2.

3.2 Efflux-Related Resistance

Examination of genome data available for *Enterococcus faecalis, S. aureus*, *S. epidermidis,* and *Streptococcus pneumoniae* reveals coding regions for several putative drug transport proteins (http://www.membranetransport.org). Many of these proteins are homologous with known multidrug transporters for which quinolones are substrates. Several of the most extensively studied Gram-positive drug pumps will be discussed in this section.

NorA is a chromosomally encoded 12 TMS *S. aureus* multidrug pump having broad substrate specificity that includes antiseptic compounds as well as quinolones (63). As are all MFS pumps, its activity is dependent on the pmf (64). Knockout mutations have revealed that NorA contributes to quinolone susceptibility in wild-type strains in that elimination of the gene results in MIC reductions for norfloxacin and ciprofloxacin (65, 66). Overexpression of *norA*, either by way of a regulatory mutation or expression from a multicopy plasmid in the laboratory, results in modest MIC increases for selected quinolones as well as many other structurally unrelated drugs, mainly hydrophobic cations (67, 68).

The understanding of *norA* regulation is incomplete. Recent work has identified the MgrA protein, which apparently binds upstream of *norA* repressing its expression (69, 70). MgrA is not a specific regulator of *norA* expression but rather is a global regulator that, in addition to affecting *norA* transcription, also affects the transcription of other pump-encoding genes (including *norB* and *norC*; see below), autolytic regulators, murein hydrolases and virulence factors such as alpha toxin, coagulase and nuclease (70–73).

NorB and NorC are two 14 TMS MFS multidrug transporters that are quite similar to each other on the basis of 70% amino acid sequence homology (74, 75). The substrate profile of NorB includes a variety of quinolones (norfloxacin, ciprofloxacin, sparfloxacin, moxifloxacin, gemifloxacin, garenoxacin, and premafloxacin), tetraphenylphosphonium bromide, cetrimide, and ethidium bromide, many of which also are substrates for NorA. NorC seems capable of effluxing a similar set of quinolone substrates with the exception of gemifloxacin. Further studies will be required to elucidate the reason(s) for this difference, but it may be related to differences in substrate binding sites. Transcriptional profiling experiments have shown that MgrA represses the expression of *norC*, but augments that of *norB* (73).

A novel *S. aureus* 14 TMS MFS multidrug efflux pump, MdeA, was recently described (76). When overexpressed in *S. aureus* MdeA confers resistance to an intriguing array of substrates including norfloxacin, ethidium bromide, benzalkonium chloride, virginiamycin, novobiocin, fusidic acid and augments EtBr efflux (76, 77). Expression of *mdeA* in wild-type strains is low, but spontaneous mutants having increased transcription are selectable in vitro. These mutants, which have reduced susceptibility to MdeA substrates, were found to have mutations in the *mdeA* promoter but further details regarding the regulation of *mdeA* expression are not available.

Although not considered a human pathogen, several multidrug transporters of *Bacillus subtilis* have been extensively studied and have contributed greatly to our knowledge of the regulation and function of MFS proteins. Bmr is a 12 TMS MFS MDR transporter having 44% amino acid identity with NorA and a similar substrate profile (78, 79). The expression of *bmr* is regulated by the binding to its promoter of BmrR, a transcriptional activator protein encoded by a gene immediately downstream from *bmr* (80). The crystal structure BmrR in the presence and absence of substrates has been solved and has revealed that Bmr substrates bind to BmrR via hydrophobic and electrostatic interactions, which in turn facilitate BmrR binding to the *bmr* promoter and induction of *bmr* transcription (81).

Blt is a second 12 TMS MFS MDR transporter of *B. subtilis* that has a similar substrate profile to those of NorA and Bmr (82). The expression of *blt* is enhanced in a similar manner to that of *bmr* by the binding of the transcriptional activator BltR (encoded by *bltR*, found immediately upstream of *blt*) to the *blt* promoter. This binding is thought to be improved by the interaction of substrates with BltR, although the specific activator substrates have not been identified. Interestingly, *blt* is not expressed in wild-type cells.

In addition to the specific regulators of *bmr* and *blt* transcription just described, the expression of these genes also is

affected by MtaN, a global transcriptional regulator that interacts with the *bmr* and *blt* promoters stimulating their transcription (83). MtaN consists of the N-terminal 109 residues of a larger protein, Mta (257 residues); the intact parent protein does not activate *bmr* or *blt* transcription. It is hypothesized that upon interacting with an inducer (as yet unidentified), the N- and C-terminal domains of Mta are functionally separated allowing it to function as a transcriptional activator.

Bmr3 is a 14 TMS MDR pump that confers reduced susceptibility to only select quinolones and puromycin when overexpressed (84). The bmr3 gene is likely poorly expressed and does not contribute to intrinsic drug resistance because when it is disrupted the norfloxacin MIC is unchanged from that of a parent strain.

PmrA is an MFS transporter found in *S. pneumoniae* (85). Disruption of *pmrA* results in increased quinolone susceptibility and reduced efflux of ethidium bromide, indicating that at least some quinolones are substrates for this pump and that it is a multidrug transporter. The contribution of PmrA to quinolone susceptibility in clinical strains is uncertain as overexpression does not necessarily result in any change in quinolone susceptibility (86).

EmeA is a NorA homologue identified by probing the *Enterococcus faecalis* V583 genome data (87). It is a multidrug pump that can transport norfloxacin and ethidium bromide and when deleted susceptibility to acriflavine and ciprofloxacin increases, suggesting that these compounds also are substrates. The contribution of EmeA to intrinsic quinolone susceptibility in clinical isolates of *E. faecalis* is unknown.

The MATE family of efflux proteins is the most recently described and the least well characterized. MATE pumps function for the most part by an unusual sodium ion:drug antiport mechanism and have been found mainly in Gram-negative bacteria, with two examples also reported in Gram-positives (88–91). MATE family proteins are similar in size to MFS transporters and are typically arranged into 12 TMSs, but they have no sequence similarity to any MFS proteins (Fig. 2). Substrates can be variable between different MATE pumps but can include cationic dyes, aminoglycosides, anticancer agents, and quinolones. Gene inactivation studies have demonstrated that MATE pump genes can be expressed at sufficient levels to affect MICs for pump substrates in wild-type cells and along with other pumps and alternative resistance mechanisms can contribute to reduced susceptibility to clinically relevant drugs such as FQs (50).

The regulation of MATE pump expression is not well understood. The MepA pump of *S. aureus* is repressed by MepR, a MarR-like protein encoded immediately upstream of *mepA* (90, 92). MepA substrates appear to bind to MepR, reducing its binding to the *mepA* promoter resulting in augmented *mepA* expression. MepR also is autoregulatory in that it represses the expression of its own gene. However, relief of *mepR* repression in the presence of MepA substrates is much less than that observed for *mepA*. The mechanism(s) of this apparent paradox are yet to be worked out, but the end result is significant relief of *mepA* and relative maintenance of *mepR* repression, leading to increased MepA protein unimpeded by MepR when the need for detoxification exists.

Lactococcus lactis is generally not considered a human pathogen but is extensively used in the dairy industry. However, like the study of multidrug pumps in *B. subtilis*, the study of such pumps in *L. lactis* has added significantly to our knowledge of how these pumps work. At least one true infection with *L. lactis* has been described making drug pumps of this organism that are capable of effluxing quinolones, in combination with other quinolone resistance mechanisms, potentially relevant clinically (93). At this time the only pump capable of transporting quinolones in *L. lactis* is LmrA, which is unique among bacterial efflux pumps capable of transporting quinolones in that it is an ABC transporter homologous with the human multidrug transporter P-glycoprotein (94). In addition to transporting quinolones it also is capable of effluxing chemotherapeutic agents such as daunorubicin.

4 Means to Limit or Overcome Quinolone Resistance

As mentioned previously, quinolones are among the most commonly prescribed antimicrobial agents. It is not infrequent that they are used inappropriately, with an example being the prescription of levofloxacin for viral upper respiratory tract infections. Education of primary care physicians regarding the seriousness of the antimicrobial agent resistance problem in general, and that of quinolones in particular, and encouraging them to not succumb to pressure to prescribe antimicrobial treatment for infections that are most likely viral in nature will help to reduce selective pressure. The dissemination of well-conceived guidelines for the proper use of these drugs and the institution of formulary restrictions are other methods by which inappropriate quinolone use might be reduced.

Once resistance to a particular antimicrobial agent reaches a critical prevalence, the utility of that drug becomes severely compromised. Most often, alternative therapy will be prescribed. Much work has been done on the development of compounds that block multidrug efflux pumps of both Gram-negative and -positive organisms, many of which have quinolones as substrates (efflux pump inhibitors, or EPIs) (95). Increased efflux often is the first step along the pathway towards high-level quinolone resistance and inhibition of this process may prevent such mutants from appearing. In addition, if efflux is the only mechanism of quinolone resis-

tance the combination of such a drug with EPI may result in the recovery of clinically useful activity of that drug. It has been shown in vitro that target-based resistance mutations occur much less frequently when an EPI is present in addition to the quinolone (6, 7, 96). Recently, an IND was filed to study the combination of an EPI (MP-601,205) with a quinolone for therapy of pulmonary infections in patients with cystic fibrosis. This will be the first clinical trial involving an EPI and its results are anxiously awaited.

References

1. Lescher GY, Froelich ED, Gruet MD, Bailey JH, Brundage RP. 1,8 naphthyridine derivatives: a new class of chemotherapy agents. J Med Pharm Chem 1962;5:1063–1068
2. Blum MD, Graham DJ, McCloskey CA. Temafloxacin syndrome: review of 95 cases. Clin Infect Dis 1994;18:946–950
3. Stahlmann R. Clinical toxicological aspects of fluoroquinolones. Toxicol Lett 2002;127:269–277
4. Ball P. Adverse drug reactions: implications for the development of fluoroquinolones. J Antimicrob Chemother 2003;51(Suppl S1): 21–27
5. Drlica K. Mechanism of fluoroquinolone action. Curr Opin Microbiol 1999;2:504–508
6. Markham PN, Neyfakh AA. Inhibition of the multidrug transporter NorA prevents emergence of norfloxacin resistance in *Staphylococcus aureus*. Antimicrob Agents Chemother 1996;40:2673–2674
7. Markham PN. Inhibition of the emergence of ciprofloxacin resistance in *Streptococcus pneumoniae* by the multidrug efflux inhibitor reserpine. Antimicrob Agents Chemother 1999;43:988–989
8. Lomovskaya O, Lee A, Hoshino K, et al. Use of a genetic approach to evaluate the consequences of inhibition of efflux pumps in *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 1999;43:1340–1346
9. Poole K. Efflux-mediated resistance to fluoroquinolones in gram-positive bacteria and the mycobacteria. Antimicrob Agents Chemother 2000;44:2595–2599
10. Jacobs MR. Fluoroquinolones as chemotherapeutic agents against mycobacterial infections. Curr Pharm Des 2004;10:3213–3220
11. Niedermann MS. Principles of appropriate antibiotic use. Int J Antimicrob Agents 2005;26(Suppl 3):S170–S175
12. Martínez-Martínez L, Pascual A, Jacoby GA. Quinolone resistance from a transferable plasmid. Lancet 1998;351:797–799
13. Gellert M, Mizuuchi K, O'Dea MH, Nash HA. DNA gyrase: and enzyme that introduces negative superhelical turns into DNA. Proc Natl Acad Sci USA 1976;73:3872–3876
14. Drlica K, Zhao X. DNA gyrase, topoisomerase IV, and the 4-quinolones. Microbiol Mol Biol Rev 1997;61:377–392
15. Gellert M, Mizuuchi K, O'Dea MH, Itoh T, Tomizawa JI. Nalidixic acid resistance: a second genetic character involved in DNA gyrase activity. Proc Natl Acad Sci USA 1977; 74:4772–4776
16. Kato J, Nishimura Y, Imamura R, Niki H, Hiraga S, Suzuki H. New topoisomerase essential for chromosome segregation in *E. coli*. Cell 1990;63:393–404
17. Ullsperger C, Cozzarelli N. Contrasting enzymatic activities of topoisomerase IV and DNA gyrase from *Escherichia coli*. J Biol Chem 1996;271:31549–31555
18. Peng H, Marians KJ. The interaction of *Escherichia coli* topoisomerase IV with DNA. J Biol Chem 1995;270:25286–25290
19. Khodursky AB, Zechiedrich EL, Cozzarelli NR. Topoisomerase IV is a target of quinolones in *Escherichia coli*. Proc Natl Acad Sci USA 1995;92:11801–11805
20. Hoshino K, Kitamura A, Morrissey I, Sato K, Kato J, Ikeda H. Comparison of inhibition of *Escherichia coli* topoisomerase IV by quinolones with DNA gyrase inhibition. Antimicrob Agents Chemother 1994;38:2623–2627
21. Khodursky AB, Cozzarelli NR. The mechanism of inhibition of topoisomerase IV by quinolone antibacterials. J Biol Chem 1998;273:27668–27677
22. Kampranis SC, Maxwell A. Conformational changes in DNA gyrase revealed by limited proteolysis. J Biol Chem 1998;273: 22606–22614
23. Krueger S, Zaccai G, Wlodawer A, et al. Neutron and light-scattering studies of DNA gyrase and its complex with DNA. J Mol Biol 1990;211:211–220
24. Marians KJ, Hiasa H. Mechanism of quinolone action. A drug-induced structural perturbation of the DNA preceded strand cleavage by topoisomerase IV. J Biol Chem 1997;272:9401–9409
25. Hiasa H, Yousef DO, Marians KJ. DNA strand cleavage is required for replication fork arrest by a frozen topoisomerase-quinolone-DNA ternary complex. J Biol Chem 1996;271:26424–26429
26. Jacoby GA. Mechanisms of resistance to quinolones. Clin Infect Dis 2005;41(Suppl 2):S120–S126
27. Yamagishi J, Kojima T, Oyamada Y, et al. Alterations in the DNA topoisomerase IV *grlA* gene responsible for quinolone resistance in *Staphylococcus aureus*. Antimicrob Agents Chemother 1996;40:1157–1163
28. Schmitz F-J, Higgins PG, Mayer S, Fluit AC. Activity of quinolones against gram-positive cocci: mechanisms of drug action and bacterial resistance. Eur J Clin Microbiol Infect Dis 2002;21: 647–659
29. Blanche F, Cameron B, Bernard FX, et al. Differential behaviors of *Staphylococcus aureus* and *Escherichia coli* type II DNA topoisomerases. Antimicrob Agents Chemother 1996;40:2714–2720
30. Ruiz J. Mechanisms of resistance to quinolones: target alterations, decreased accumulation and DNA gyrase protection. J Antimicrob Chemother 2003;51:1109–1117
31. Hooper DC. Mechanisms of quinolone resistance. In: Hooper DC, Rubenstein E, eds. *Quinolone Antimicrobial Agents*. Washington, DC: American Society for Microbiology, 2003, pp. 41–67
32. Horowitz DS, Wang JC. Mapping the active site tyrosine of *Escherichia coli* DNA gyrase. J Biol Chem 1987;262:5339–5344
33. Chapman JS, Georgopapadokou NH. Routes of quinolone permeation in *Escherichia coli*. Antimicrob Agents Chemother 1988;32:438–442
34. Nikaido H. Molecular basis of bacterial outer membrane permeability revisited. Microbiol Mol Biol Rev 2003;67:593–656
35. Cohen SP, Hooper DC, Wolfson JS, Souza KS, McMurry LM, Levy SB. Endogenous active efflux of norfloxacin in susceptible *Escherichia coli*. Antimicrob Agents Chemother 1988;32: 1187–1191
36. Wiedemann B, Heisig P. Mechanisms of quinolone resistance. Infection 1994;22(Suppl 2):S73–S79
37. Alekshun MN, Levy SB. Regulation of chromosomally mediated multiple antibiotic resistance: the *mar* regulon. Antimicrob Agents Chemother 1997;41:2067–2075
38. Chou JH, Greenberg JT, Demple B. Posttranscriptional repression of *Escherichia coli* OmpF protein in response to redox stress: positive control of the *micF* antisense RNA by the *soxRS* locus. J Bacteriol 1993;175:1026–1031
39. Yoshimura F, Nikaido H. Permeability of *Pseudomonas aeruginosa* outer membrane to hydrophilic solutes. J Bacteriol 1982;152: 636–642
40. Roberts MC. Update on acquired tetracycline resistance genes. FEMS Microbiol Lett 2005;245:195–203
41. Poole K. Efflux-mediated resistance to fluoroquinolones in gram-negative bacteria. Antimicrob Agents Chemother 2000;44: 2233–2241

42. Okusu H, Ma D, Nikaido H. AcrAB efflux pump plays a major role in the antibiotic resistance phenotype of *Escherichia coli* multiple-antibiotic-resistance (Mar) mutants. J Bacteriol 1996;178:306–308
43. Zgurskaya HI, Nikaido H. Cross-linked complex between oligomeric periplasmic lipoprotein AcrA and the inner-membrane-associated multidrug efflux pump AcrB from *Escherichia coli*. J Bacteriol 2000;182:4264–4267
44. Alekshun MN, Levy SB. The *mar* regulon: multiple resistance to antibiotics and other toxic chemicals. Trends Microbiol 1999;7:410–413
45. Murakami S, Nakashima R, Yamashita E, Yamaguchi A. Crystal structure of bacterial multidrug transporter AcrB. Nature 2002;419:587–593
46. Elkins CA, Nikaido H. Substrate specificity of the RND-type multidrug efflux pumps AcrB and AcrD of *Escherichia coli* is determined predominantly by two large periplasmic loops. J Bacteriol 2002;184:6490–6498
47. Li X-Z, Livermore DM, Nikaido H. Role of efflux pumps in intrinsic resistance of *Pseudomonas aeruginosa*: resistance to tetracycline, chloramphenicol, and norfloxacin. Antimicrob Agents Chemother 1994;38:1732–1741
48. Alonso A, Martinez JL. Cloning and characterization of SmeDEF, a novel multidrug efflux pump from *Stenotrophomonas maltophilia*. Antimicrob Agents Chemother 2000;45:1879–1881
49. Morita Y, Kodama K, Shiota S, et al. NorM, a putative multidrug efflux protein, of *Vibrio parahaemolyticus* and its homolog in *Escherichia coli*. Antimicrob Agents Chemother 1998;42: 1778–1782
50. Miyamae S, Ueda O, Yoshimura F, Hwang J, Tanaka Y, Nikaido H. A MATE family multidrug efflux transporter pumps out fluoroquinolones in *Bacteriodes thetaiotaomicron*. Antimicrob Agents Chemother 2001;45:3341–3346
51. Colmer JA, Fralick JA, Hamood AN. Isolation and characterization of a putative multidrug efflux pump from *Vibrio cholerae*. Mol Microbiol 1998;27:63–72
52. Tran JH, Jacoby GA. Mechanism of plasmid-mediated quinolone resistance. Proc Natl Acad Sci USA 2002;99:5638–5642
53. Tran JH, Jacoby GA, Hooper DC. Interaction of the plasmid-encoded quinolone resistance protein Qnr with *Escherichia coli* DNA gyrase. Antimicrob Agents Chemother 2005;49: 118–125
54. Tran JH, Jacoby GA, Hooper DC. Interaction of the plasmid-encoded quinolone resistance protein QnrA with *Escherichia coli* topoisomerase IV. Antimicrob Agents Chemother 2005;49: 3050–3052
55. Wang M, Tran JH, Jacoby GA, Zhang Y, Wang F, Hooper DC. Plasmid-mediated quinolone resistance in clinical isolates of *Escherichia coli* from Shanghai, China. Antimicrob Agents Chemother 2003;47:2242–2248
56. Wang M, Sahm DF, Jacoby GA, Hooper DC. Emerging plasmid-mediated quinolone resistance associated with the *qnr* gene in *Klebsiella pneumoniae* clinical isolates in the United States. Antimicrob Agents Chemother 2004;48:1295–1299
57. Robicsek A, Sahm DF, Strahilevitz J, Jacoby GA, Hooper DC. Broader distribution of plasmid-mediated quinolone resistance in the United States. Antimicrob Agents Chemother 2005;49: 3001–3003
58. Jacoby GA, Walsh KE, Mills DM, et al. *qnrB*, another plasmid-mediated gene for quinolone resistance. Antimicrob Agents Chemother 2006;50:1178–1182
59. Martens R, Wetzstein H-G, Zadrazil F, Capelari M, Hoffman P, Schmeer N. Degradation of the fluoroquinolone enrofloxacin by wood-rotting fungi. Appl Environ Microbiol 1996;62:4206–4209
60. Wetzstein H-G, Stadler M, Tichy H-V, Dalhoff A, Karl W. Degradation of ciprofloxacin by basidiomyectes and identification of metabolites generated by the brown rot fungus *Gloeophyllum striatum*. Appl Environ Microbiol 1999;65:1556–1563
61. Robicsek A, Strahilevitz J, Jacoby GA, et al. Fluoroquinolone-modifying enzyme: a new adaptation of a common aminoglycoside acetyltransferase. Nat Med 2006;12:83–88
62. Ng EY, Trucksis M, Hooper DC. Quinolone resistance mutations in topoisomerase IV: relationship to the *flqA* locus and genetic evidence that topoisomerase IV is the primary target and DNA gyrase the secondary target of fluoroquinolones in *Staphylococcus aureus*. Antimicrob Agents Chemother 1996;40:1881–1888
63. Neyfakh AA, Borsch CM, Kaatz GW. Fluoroquinolone resistance protein NorA of *Staphylococcus aureus* is a multidrug efflux transporter. Antimicrob Agents Chemother 1993;37:128–129
64. Yu J-L, Grinius L, Hooper DC. NorA functions as a multidrug efflux protein in both cytoplasmic membrane vesicles and reconstituted proteoliposomes. J Bacteriol 2002;184:1370–1377
65. Hsieh P-C, Siegel SA, Rogers B, Davis D, Lewis K. Bacteria lacking a multidrug pump: a sensitive tool for drug discovery. Proc Natl Acad Sci USA 1998;95:6602–6606
66. Kaatz GW, Seo SM, O'Brien L, Wahiduzzaman M, Foster TJ. Evidence for the existence of a multidrug efflux transporter distinct from NorA in *Staphylococcus aureus*. Antimicrob Agents Chemother 2000;44:1404–1406
67. Yoshida H, Bogaki M, Nakamura S, Ubukata K, Konno M. Nucleotide sequence and characterization of the *Staphylococcus aureus norA* gene, which confers resistance to quinolones. J Bacteriol 1990;172:6942–6949
68. Kaatz GW, Seo SM, Ruble CA. Efflux-mediated fluoroquinolone resistance in *Staphylococcus aureus*. Antimicrob Agents Chemother 1993;37:1086–1094
69. Kaatz GW, Thyagarajan RV, Seo SM. Effect of promoter region mutations and *mgrA* overexpression on transcription of *norA*, which encodes a *Staphylococcus aureus* multidrug efflux transporter. Antimicrob Agents Chemother 2005;49:161–169
70. Ingavale SS, Van Wamel W, Cheung AL. Characterization of RAT, and autolysis regulator in *Staphylococcus aureus*. Mol Microbiol 2003;48:1451–1466
71. Luong TT, Newell SW, Lee CY. *mgr*, a novel global regulator in *Staphylococcus aureus*. J Bacteriol 2003;185:3703–3710
72. Ingavale S, Van Wamel W, Luong TT, Lee CY, Cheung AL. Rat/MgrA, a regulator of autolysis, is a regulator of virulence genes in *Staphylococcus aureus*. Infect Immun 2005;73:1423–1431
73. Luong TT, Dunman PM, Murphy E, Projan SJ, Lee CY. Transcription profiling of the *mgrA* regulon in *Staphylococcus aureus*. J Bacteriol 2006;188:1899–1910
74. Truong-Bolduc Q-C, Dunman PM, Strahilevitz J, Projan SJ, Hooper DC. MgrA is a multiple regulator of two new efflux pumps in *Staphylococcus aureus*. J Bacteriol 2005;187:2395–2405
75. Truong-Bolduc Q-C, Strahilevitz J, Hooper DC. NorC, a new efflux pump regulated by MgrA of *Staphylococcus aureus*. Antimicrob Agents Chemother 2006;50:1104–1107
76. Huang J, O'Toole P, Shen W. Novel chromosomally-encoded multidrug efflux transporter MdeA in *Staphylococcus aureus*. Antimicrob Agents Chemother 2004;48:909–917
77. Yamada Y, Shiota S, Mizushima T, Kuroda T, Tsuchiya T. Functional gene cloning and characterization of MdeA, a multidrug efflux pump from *Staphylococcus aureus*. Biol Pharm Bull 2006;29:801–804
78. Neyfakh AA, Bidnenko VE, Chen LB. Efflux-mediated multidrug resistance in *Bacillus subtilis*: similarities and dissimilarities with the mammalian system. Proc Natl Acad Sci USA 1991;88:4781–4785
79. Neyfakh AA. The multidrug efflux transporter of *Bacillus subtilis* is a structural and functional homologue of the *Staphylococcus aureus* NorA protein. Antimicrob Agents Chemother 1992;36:484–485
80. Ahmed M, Borsch CM, Taylor SS, Vazquez-Laslop N, Neyfakh AA. A protein that activates expression of a multidrug efflux trans-

porter upon binding the transporter substrates. J Biol Chem 1994;269:28506–28513
81. Zheleznova EE, Markham PN, Neyfakh AA, Brennan RG. Structural basis of multidrug recognition by BmrR, a transcription activator of a multidrug transporter. Cell 1999;96:353–362
82. Ahmed M, Lyass L, Markham PN, Taylor SS, Vazquez-Laslop N, Neyfakh AA. Two highly similar multidrug transporters of *Bacillus subtilis* whose expression is differentially regulated. J Bacteriol 1995;177:3904–3910
83. Baranova NN, Danchin A, Neyfakh AA. Mta, a global MerR-type regulator of the *Bacillus subtilis* multidrug-efflux transporters. Mol Microbiol 1999;31:1549–1559
84. Ohki R, Murata M. *bmr3*, a third multidrug transporter gene of *Bacillus subtilis*. J Bacteriol 1997;179:1423–1427
85. Gill MJ, Brenwald NP, Wise R. Identification of an efflux pump gene, *pmrA*, associated with fluoroquinolone resistance in *Streptococcus pneumoniae*. Antimicrob Agents Chemother 1999;43:187–189
86. Piddock LJV, Johnson MM, Simjee S, Pumbwe L. Expression of efflux pump geme *pmrA* in fluoroquinolone-resistant and -susceptible clinical isolates of *Streptococcus pneumoniae*. Antimicrob Agents Chemother 2002;46:808–812
87. Jonas BM, Murray BE, Weinstock GM. Characterization of *emeA*, a *norA* homologue and multidrug resistance efflux pump, in *Enterococcus faecalis*. Antimicrob Agents Chemother 2001;45:3574–3579
88. Borges-Walmsley MI, McKeegan KS, Walmsley AR. Structure and function of efflux pumps that confer resistance to drugs. Biochem J 2003;376:313–338
89. Dridi L, Tankovic J, Petit JC. CdeA of *Clostridium difficile*, a new multidrug efflux transporter of the MATE family. Microb Drug Resist 2004;10:191–196
90. Kaatz GW, McAleese F, Seo SM. Multidrug resistance in *Staphylococcus aureus* due to overexpression of a novel multidrug and toxin extrusion (MATE) transport protein. Antimicrob Agents Chemother 2005;49:1857–1864
91. McAleese F, Petersen P, Ruzin A, et al. A novel MATE family efflux pump contributes to the reduced susceptibility of laboratory-derived *Staphylococcus aureus* mutants to tigecycline. Antimicrob Agents Chemother 2005;49:1865–1871
92. Kaatz GW, DeMarco CE, Seo SM. MepR, a repressor of the *Staphylococcus aureus* MATE family multidrug efflux pump MepA, is a substrate-responsive regulatory protein. Antimicrob Agents Chemother 2006;50:1276–1281
93. Akhaddar A, El Mostarchid B, Gazzaz M, Boucetta M. Cerebellar abscess due to *Lactococcus lactis*. A new pathogen. Acta Neurochir 2002;144:305–306
94. Poelarends GJ, Mazurkiewicz P, Konings WN. Multidrug transporters and antibiotic resistance in *Lactococcus lactis*. Biochim Biophys Acta 2002;1555:1–7
95. Kaatz GW. Bacterial efflux pump inhibition. Curr Opin Investig Drugs 2005;6:191–198
96. Lomovskaya O, Warren MS, Lee A, et al. Identification and characterization of multidrug resistance efflux pumps in *Pseudomonas aeruginosa*: novel agents for combination therapy. Antimicrob Agents Chemother 2001;45:105–116